THE EFFECT OF COCOZEN ON EXTRA-MEDIA THICKNESS AND ARTERIAL STIFFNESS IN MIDDLE-AGED AND OLDER ADULTS

A THESIS

SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF SCIENCE

BY

SPENCER MICHAEL ROMANOWSKI

DR. BRAD FLEENOR - ADVISOR

BALL STATE UNIVERSITY

MUNCIE, INDIANA

MAY 2020

Table of Contents

Acknowledgments

First, I want to thank Dr. Fleenor and Nick Carlini. From the beginning, you both took me under your wing and allowed me to learn ultrasound and be a part of COCOZEN. For that I am grateful, I appreciate all the conversations that made me think harder about not just science, but life as well. I appreciate all the encouraging words and constant belief in my abilities. You made me better here. Thank you for that.

Second, to the other members of my thesis committee, Dr. Harber, and Dr. Kistler. Thank you for working with me throughout this entire process. Dr. Harber, thank you for accepting me into the program and being patient with me. I greatly appreciate it.

Third, I want to thank my parents, my sisters Claire and Molly, my brother Max, my brother-in-law Cam, and best friend, Noah. Thank you for working with me and loving me throughout this rewarding process. Thank you for your constant emotional, spiritual, and financial support.

I would like to thank my fellow classmates, HPL staff, hospital co-workers, and Taylor University professors: Olivia Jones, McKenzie Metz, Matt Riccardi, Malvina Shourki, Hannah Remington, Becky Collins, Nicole Koontz, Todd Trappe, Scott Trappe, Mitchell Whaley, Leonard Kaminsky, Katrina Riggin, Anna Taylor, Kelsie Ostojic, Matt Renfrow, and Erik Hayes. Thank you for all of your help and support during my time at Ball State.

I want to thank my Lord and Savior Jesus Christ for without you, nothing here has any purpose. Lastly, Annie, thank you for dealing with my constant change of emotion and loving me throughout my time in grad school. I cannot wait to marry you.

THESIS: The Effect of COCOZEN on EMT and Arterial Stiffness in Middle-Aged and Older Adults.

STUDENT: Spencer M. Romanowski

DEGREE: Master of Science

COLLEGE: College of Health

DATE: May 2020

PAGES: 42

Introduction: It is known that aging results in an increased risk for cardiovascular disease (CVD) related events. Age-related CVD events are associated with arterial stiffening independent of traditional risk factors. Extra-media thickness (EMT) is a novel structural component of the carotid artery composed of perivascular adipose tissue (PVAT), arterial adventitia, and the interstitial wall that may be associated with age-related arterial stiffness. However, it is unknown what affect dietary interventions have on EMT and arterial stiffness. **Purpose**: The purpose of this study was to determine if an 8-week COCOZEN intervention decreases age-related EMT and arterial stiffness. **Methods:** Sixteen participants with a mean age of 54.81 ± 7.0 years old were randomly assigned to either COCOZEN or the Placebo for 8 weeks. Carotid artery stiffness, and carotid and brachial blood pressure were acquired before and after the intervention. A two-way repeated-measures analysis of variance (ANOVA) was used to analyze changes over time, an unpaired one-tailed t-test was utilized to determine percent (%) change on blood pressure (BP), between groups, and a correlation at baseline was used to determine associations between EMT and arterial stiffness. **Results:** Carotid systolic blood pressure (CSBP; mmHg) and brachial systolic blood pressure (BSBP; mmHg) were decreased in the COCOZEN group demonstrated, with a 6.57% and 6.64% decrease ($p=0.039$, 0.022), respectively. There were no differences between groups for EMT or carotid compliance ($p \geq 0.05$). Carotid distensibility (10^{-3}/kPa) and β-stiffness (AU) decreased 0.005 ± 0.002 to 0.003 ± 0.002 and increased 3.13 ± 0.40 to 3.40 ± 0.34, respectively, in the placebo group ($p \leq 0.05$). **Conclusion:** COCOZEN lowered CSBP and BSBP, indicating this novel nutraceutical compound may lower CV risk.

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide (1). With increasing prevalence of CVD nationally and globally, the necessity to understand risk factors associated with this condition become clinically relevant (1,2). Aging results in an increased risk for CVD related events, which is associated with stiffening of the large elastic arteries, including the aorta and carotid artery (3,4,5,6). Arterial stiffening predicts CVD-related events independent of traditional risk factors and is associated with structural changes in the artery (2,3,4,5,6).

With aging, arterial stiffening increases as a result of structural changes in the artery (4,6). The stiffening of the artery and ensuing structural changes are associated with inflammation within the vasculature (3,4,6). After persistent exposure to inflammation, specific structural components change to promote arterial stiffness (3,6,7). These structures include extra-media thickness (EMT), which is a composite measure that includes perivascular adipose tissue (PVAT), arterial adventitia and the interstitial wall of the carotid artery and intima-media thickness (IMT) which encompasses the tunica intima and media of the artery (3,6,7). Research has demonstrated that increases in EMT is associated with increased inflammation that contributes to stiffening (7,8,9,10,11). Understanding how structural components contribute to stiffness as well as other lifestyle choices is essential in determining the role of the EMT on and potential association with arterial stiffness.

Lifestyle choices, such as diet contribute to arterial stiffness and inflammation (2,6). Dietary interventions have demonstrated that anti-inflammatory compounds can have a positive effect on vascular health (12,13,14,15). One such compound is COCOZEN, which is a coconut-derived substance that has shown to have anti-inflammatory properties (12,13). COCOZEN has previously been utilized in other cultures as an anti-inflammatory agent (12,13,15). However, it has limited usage in specific dietary interventions, and the effect of COCOZEN on EMT and arterial stiffness is unknown.

Therefore, the aim of this study was to determine if an 8-week COCOZEN intervention decreases age-related EMT and arterial stiffness, and if EMT is associated with arterial stiffness. We hypothesized that adherence to COCOZEN over 8-weeks would decrease EMT and lower arterial stiffness in middle-aged and older adults compared to the placebo, and that increased EMT would be associated with arterial stiffness.

Methods

Included within this study were 16 males and females, who were free of any known clinical disease. Participants were between the ages of 40-80 years old and were absent of diagnosed hypertension and/or anti-hypertensive medications. Participants were excluded if they exhibited one or more of the following: a body mass index (BMI) < 18 kilogram/meters^2 (kg/m^2) or > 35 kg/m^2, resting brachial blood pressure of greater than 160/100 millimeters of mercury (mmHg), a current smoker, known clinical disease, an abnormal resting 12-lead electrocardiogram (ECG), previous angina, alcohol dependence or abuse, more than three days of self-reported vigorous activity per week, or self-reported allergies to nuts or coconuts.

Procedures

This study was an 8-week, double-blind, randomized control trial. After qualifying for participation, participants were randomly assigned to either the COCOZEN or placebo group. This study included two testing sessions, the pre-test at week 0 and the post-test at week 8. The pre-test encompassed an informed consent and health history questionnaire. Participants were instructed to refrain from caffeine, alcohol, and exercise 24 hours prior to the pre-test and were required to perform an overnight fast. The supplement or placebo was administered in a 28-day pill tray and given to each participant. Three pills at 500 milligrams (mg) each (total 1500 mg) per day were taken and recorded on a log sheet. Participant adherence to the supplement was 99%. The post-test at week 8 followed the same procedures as the pre-test.

Anthropometrics and Blood Draw

Baseline measurements included basic anthropometrics, encompassing height, weight, and body fat percentage measured with dual-energy X-ray absorptiometry (DEXA). Additionally, a blood draw with a full lipid profile and glucose was conducted. All anthropometric measures were standardized following the American College of Sports Medicine (ACSM) (2).

Resting Cardiovascular Measurements

Participants were seated approximately for five-minutes before brachial blood pressure was measured two times and averaged (2). Thereafter, blood pressure measurements at the carotid and radial pulse were performed and conducted by the same technician with a SphygmoCor system tonometer (7) Participants were instructed to lie down in the supine position for approximately 10 minutes (7). Upon which, the pressure waveform measurements were obtained on the left radial pulse and left common carotid artery (LCCA) (7). A minimum of three measurements were taken within 4 mmHg of each other. The carotid pressure measurement on the LCCA was measured twice, once before ultrasound and then after.

Ultrasound Measurements: EMT

Ultrasound was performed with the participant in the supine position. A three-lead ECG was attached, and a longitudinal measurement of the right common carotid artery (RCCA) was measured (7). After capturing videos approximately 15-20 seconds long, EMT was measured on the near wall on the QRS-complex during diastole, 10-15 mm proximal of the carotid bulb in the same image (16,17,18,19). Afterward, manual measurement of the EMT was performed nine times over three cardiac cycles in the same location.

IMT and Diameter

IMT was measured with CV suite. The software analyzed the captured video of the longitudinal view of the RCCA. After the technician manually chose a specific region of interest on the far wall of the artery, the software auto-calculated the measurement. The diameter was obtained in the identical image from the same region of interest and auto-calculates the IMT on the far wall to the IMT on the near wall during systole and diastole, respectively. Upon which, the average diameters were calculated over four to five cardiac cycles.

β-stiffness Index

Carotid β-stiffness was defined as Ln (P_{max}/P_{min})/[D_{max}-D_{min})/D_{min}] where, P represents pressure and D, diameter (7). The pressure component used for β-stiffness was performed with a SphygmoCor system tonometer on the LCCA (7). After three measurements of carotid blood pressure are obtained, the average systolic and diastolic carotid pressures were acquired and inputted into the P-max and P-min variables in the equation, where P-max represents the maximum systolic pressure and P-min represents the minimum diastolic pressure (7). The average systolic and diastolic measurements of the diameters were entered in the D-max and D-min portion of the equation, where D-max represents the maximum systolic diameter and D-min represents the minimum diastolic diameter (7).

Carotid Compliance and Distensibility

Compliance and distensibility were calculated as $\pi D_{min} * \Delta D / 2 * \Delta P$ and $2 * [(\Delta D / D_{min}) / \Delta P]$, respectively (20,21). Carotid distensibility and compliance were calculated with the same diameters and carotid pressures utilized for β-stiffness. The ΔD and ΔP represents the change in diameter and pressure respectively (7,20,21).

Statistical Analysis

Statistical analysis was conducted through GraphPad Prism™. Baseline characteristics between groups were analyzed through an unpaired two-tailed t-test. Measures of EMT, IMT, and stiffness were analyzed through a 2-way repeated-measures analysis of variance (ANOVA) with an uncorrected fisher's Least Significant Difference post hoc. For %change in blood pressure an unpaired one-tailed t-test was utilized and at baseline a correlation was conducted to determine associations between EMT and arterial stiffness. Group data are presented as mean ± standard deviation, with statistical significance set at $p \leq 0.05$.

Results

There were 16 participants included within this study. At baseline, there were no significant differences between age, anthropometric measurements, and/or carotid/brachial blood pressure ($p \geq 0.05$). Descriptive characteristics between groups at baseline are presented in Table 1.

Figure 1 and 2 demonstrates COCOZEN lowered both CSBP and BSBP from pre to post for the COCOZEN group with an 6.57% and 6.64% decrease, respectively ($p \leq 0.05$, both).

COCOZEN did not change EMT or IMT over the duration of the study (Table 2); however, IMT increased in the placebo group ($p \leq 0.05$). Carotid compliance, distensibility and β-stiffness was not different for the COCOZEN group ($p \geq 0.05$). Carotid distensibility and β-stiffness decreased and increased, respectively for the placebo ($p \leq 0.05$, Table 3). Additionally, a main effect on time was demonstrated for carotid diastolic blood pressure (data not presented, $p \leq 0.05$). A correlation between EMT, the three measures of arterial stiffness (β-stiffness, carotid compliance, and carotid distensibility) was conducted, and results demonstrated that there was no relationship between EMT, β-stiffness, carotid compliance, or carotid distensibility at baseline ($p \geq 0.05$) (Table 4).

Table 1: Descriptive characteristics and standard deviations between groups at baseline.

Baseline	Pre	
Value	**COCOZEN**	**Placebo**
Age (years)	**54.63 ± 8.23**	**55.00 ± 6.12**
Weight (kg)	**79.17 ± 12.44**	**80.10 ± 12.17**
Height (centimeters)	**171.77 ± 8.14**	**172.48 ± 5.96**
BMI kg/m^2	**26.74 ± 3.08**	**27.05 ± 4.84**
Brachial Systolic BP (mmHg)	**117.50 ± 10.52**	**108.63 ± 12.36**
Brachial Diastolic BP (mmHg)	**71.75 ± 7.36**	**67.50 ± 7.23**
Carotid Systolic BP (mmHg)	**115.47 ± 17.28**	**105.99 ± 16.75**
Carotid Diastolic BP (mmHg)	**73.50 ± 8.67**	**72.00 ± 7.48**
Body fat (%)	**31.76 ± 8.09**	**40.00 ± 8.28**

Figure 1: CSBP mean data over the pre and post between groups (left) and percent (%) change for both groups between the pre and post (right). *p≤0.05 vs. COCOZEN Pre for CSBP; **p≤0.05 vs Placebo” for %change.

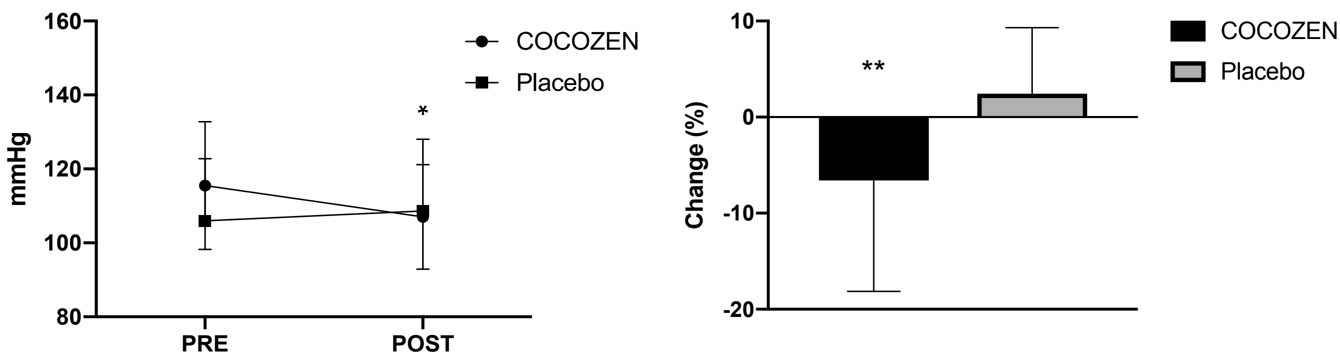


Figure 2: BSBP mean data over the pre and post between groups (left) and percent (%) change for both groups between the pre and post (right). *p≤0.05 vs. COCOZEN Pre for BSBP; **p≤0.05 vs Placebo for %change.

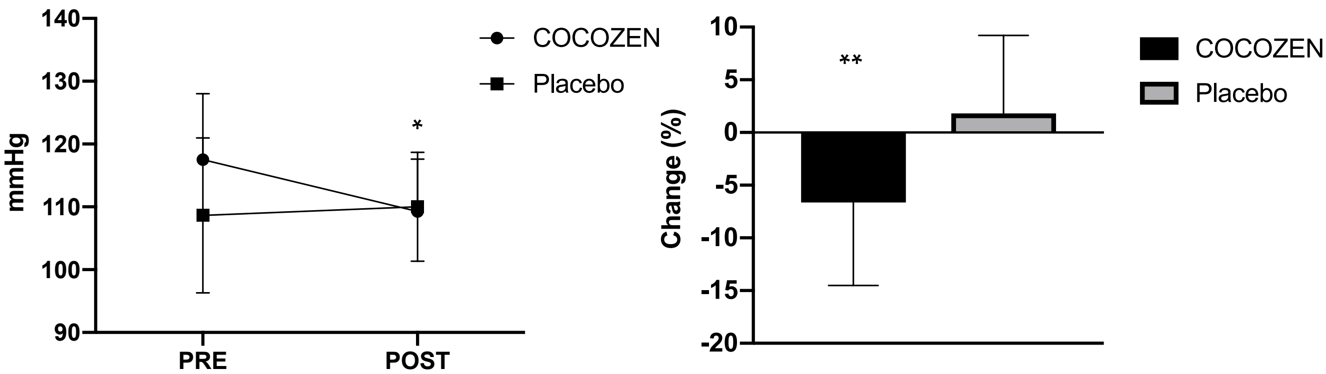


Table 2: EMT and IMT data for the pre and post between groups. *p≤0.05 vs. Placebo Pre.

	COCOZEN		Placebo	
	Pre	**Post**	**Pre**	**Post**
EMT (mm)	**0.66 ± 0.17**	**0.66 ±0.17**	**0.66 ± 0.13**	**0.66 ± 0.12**
IMT (mm)	**0.56 ± 0.11**	**0.55 ± 0.10**	**0.51 ± 0.07**	**0.55 ±0.08***

Table 3: Carotid compliance, distensibility, and β-stiffness data for the pre and post between groups. *p≤0.05 vs. Placebo Pre.

	COCOZEN		Placebo	
	Pre	**Post**	**Pre**	**Post**
Carotid Compliance (mm^2/mmHg)	**0.11 ± 0.06**	**0.13 ± 0.07**	**0.12 ± 0.04**	**0.09 ± 0.04**
Carotid Distensibility (10^{-3}/kPa)	**0.003 ± 0.001**	**0.004 ± 0.001**	**0.005 ± 0.002**	**0.003 ± 0.002***
β-Stiffness (AU)	**3.33 ± 0.45**	**3.23 ± 0.32**	**3.13 ± 0.40**	**3.40± 0.34***

mm^2/mmHg, millimeters squared divided by millimeters of mercury, 10^{-3}/kPa, ten to the negative third power divided by kilopascals, AU, arbitrary units

Table 4: EMT correlation between carotid compliance, distensibility, and β-stiffness, data at baseline.

Baseline Compared to EMT	r-value	P-value
Carotid Compliance (mm^2/mmHg)	**-0.059**	**0.827**
Carotid Distensibility (10^{-3}/kPa)	**0.167**	**0.534**
β-Stiffness (AU)	**-0.088**	**0.744**

Discussion

The effects of COCOZEN on vascular health is unknown. Arterial stiffness, a measure of vascular health increases with aging, and is related to change in vascular structures, including EMT (3,4,6). Previous literature demonstrates efficacy for anti-inflammatory dietary interventions to lower arterial stiffness, that may be related to decreases in structural components such as EMT (7,12-15). Our findings demonstrate that COCOZEN did not improve arterial stiffness but did decrease CSBP and BSBP in apparently healthy middle-aged and older adults. These findings suggest COCOZEN may be a novel intervention to lower blood pressure in middle-age/older adults and reduce future CVD risk.

Hypertension is a risk factor for CV related events (3,6,14). Thus, the importance of lowering blood pressure with aging is crucial to prevent impairments of vascular health (3,6,14). COCOZEN is a novel nutraceutical with limited research regarding its effect on vascular health (12-15). Nonetheless, research in animals has demonstrated that COCOZEN reduced mean systolic blood pressure in hypertensive rats (15). However, research for COCOZEN in humans is limited (12-15). Previous research in humans has demonstrated other dietary interventions such as, raw beet juice, to improve vascular health (12-15). As such, Asgary et al, demonstrated that both raw beet juice and cooked beet juice improved blood pressure, endothelial function, and decreased systemic inflammation in hypertensive patients (14). Though inflammation was not directly measured in the current study, similar reductions in systolic blood pressure were demonstrated in the previous study with a 4.97% and 4.02% decrease for raw beet juice and cooked beet juice, respectively (14). This is similar to our cohort’s reduction of 6.57% and 6.64% for CSBP and BSBP, respectively. Our cohort was absent of hypertension and/or any anti-hypertensive medications. This demonstrates that even in apparently healthy, non-hypertensive participants that COCOZEN has promising effects of lowering CV risk through reductions in carotid and peripheral systolic blood pressure.

Contrary to our hypothesis EMT, carotid compliance, distensibility, and β-stiffness did not change in the COCOZEN group. EMT is a structural component of the vasculature (6). Traditional risk factors including hypertension affects the structural integrity of the artery and with aging contributes to structural remodeling (3,6,22-27). Research has demonstrated that arterial stiffness and remodeling in the artery occurs over the lifespan (27). COCOZEN has been presented as a nutraceutical that can positivity impact vascular health (12,13). However, it is unknown if structural remodeling can occur with a supplement alone over an 8-week intervention. Research has indicated that aerobic exercise can reverse or delay arterial stiffness and the adventitial layer of the artery (5). To our knowledge, no literature has demonstrated in humans that structural changes with an anti-inflammatory supplement alone can occur over an 8-week period. Indicating that possibly the time of the study was not long enough for structural changes in EMT to occur or signifying structural remodeling cannot occur with a supplement alone.

Based on previous literature our rationale was that a nutraceutical supplement would facilitate a reduction in arterial stiffness (12-15). Research performed in Wistar rats (pre-treated with gentamicin) demonstrated that 20 mg/kg of bodyweight of a coconut flower sap supplement assisted in reduction of inflammatory markers in 16 days (12). The administered dosage in the previous study averaged 3 grams (g) per day for 16 days versus the current study, which averaged 1.5 g per day for 56 days. (12). Indicating that there was no relationship between arterial stiffness and COCOZEN possibly because the dosage may have not been significant enough to allow for an adequate change.

Carotid distensibility and β-stiffness decreased and increased, respectively, in the placebo group, while compliance did not change in either group. Factors associated with reductions in distensibility and increases in stiffness are increased cholesterol, body fat and/or dietary changes (6,7,12,13,14,17). However, there were no statistical differences between either measurement over time between groups. Additionally, participants were instructed to remain consistent with their daily routines, including diet and exercise. However, diet was not controlled in this study. The placebo was a dextrin extract, which is a simple sugar and derivative of starch. Thus, diet with the additive effect of the sugar derivative placebo may have affected some of the changes exhibited. Due to the novel nature of this investigation, speculations surrounding COCOZEN support an anti-inflammatory related claim (12-14). Our data support that COCOZEN supplementation potentially offset some of the possible changes associated with arterial stiffness (6,12,13). Compared to COCOZEN the placebo, did not have this effect, indicating COCOZEN potentially allowed for less fluctuation.

Our findings also indicate that no associations between EMT, and arterial stiffness (carotid compliance, distensibility, and β-stiffness) were demonstrated at baseline. Previous literature has demonstrated strong associations between these measures of stiffness and all-cause mortality (7,20,21,28-30). Research conducted by Cai et al, examined EMT and its associations to three measures of stiffness (30). Specifically, β-stiffness, carotid distensibility, and incremental modulus of elasticity in 248, 8-year old children (30). Results demonstrated that EMT was positivity correlated to all three measures of stiffness (30). Furthermore, research performed by Skilton et al, identified positive correlations between EMT and CV risk factors in 175 adults (17). It was concluded that individuals with diabetes and dyslipidemia, demonstrated an increased average EMT (0.76 mm) compared to the apparently healthy control (0.69) (17). Similar to others, our sample had an average EMT 0.66 mm at baseline. Nonetheless, limited research has demonstrated no relationship between EMT and measures of stiffness (31). Research conducted by Cai et al, demonstrated that increased EMT in high-risk adults had no significant associations for risk of a CV event (31). Our sample of 16 middle-aged and older apparently healthy adults, however, may have been too small to detect any correlations (7,17,30,31). Additionally, our sample had no known clinical disease differing our population from other literature presented, which may have contributed to the lack of correlation. Lastly, if there is no association between high-risk adults, EMT, and CV events as was presented, then the likelihood of an association of apparently healthy adults, may be unlikely (31-35).

Strengths and Limitations

Strengths of this study included that it was a double-blinded, randomized control trial, which limited any potential bias. Furthermore, there were multiple time points which allowed for repeated measurements. Limitations of this study included a small sample size of 16 participants, a relatively low supplementation dosage and a short-term intervention (8-weeks), which may have prevented an adequate time to allow for change to occur.

Conclusion

The main findings of this research project demonstrated that 1) COCOZEN lowers both CSBP and BSBP in apparently healthy middle-aged and older adults; 2) COCOZEN did not impact EMT or arterial stiffness compared to the placebo, and; 3) There were no associations between EMT and arterial stiffness. Thus, COCOZEN may lower CV risk by decreasing systolic blood pressure in apparently healthy, middle-aged adults.

Disclaimers: This research study was funded by Akay Flavours & Aromatics Pvt.

References

1. **Fuster, V., Mearns, B.** The CVD paradox: mortality vs. prevalence. *Nature Reviews Cardiology*. 2009; 6, 669.
2. **Riebe D, Ehrman J, Liguori G, Magal M.** *ACSM's Guidelines for Exercise Testing and Prescription*. 10th ed. Wolters Kluwer; 2016.1-451.
3. **Lyle AN, Raaz U, et al.** Killing me un-softly: Causes and mechanisms of arterial stiffness Recent Highlights of ATVB: *Journal of Arteriosclerosis Thrombosis Vascular Biology*. 2017; 37:1–22.
4. **Mitchell GF, Hwang S, Vasan RS, et al.** Arterial stiffness and cardiovascular events: The Framingham Heart Study. *Journal of Circulation*. 2011; 121: 505–11.
5. **Fleenor BS, Marshall KD, Durrant JR, Lesniewski LA, Seals DR.** Arterial stiffening with ageing is associated with transforming growth factor- β 1-related changes in adventitial collagen : reversal by aerobic exercise. *Journal of Physiology*. 2010; 20: 3971–82.
6. **Fleenor BS, Berrones AJ.** *Arterial Stiffness: Implications and Interventions*. Springer Briefs in Physiology. 2015: 1-67.
7. **Lefferts WK, Sperry SD, Jorgensen RS, Kasprowicz AG, Skilton MR, Figueroa A, and Heffernan KS.** Carotid stiffness, extra-media thickness, and, visceral adiposity in young adults. *Journal of Atherosclerosis.* 2017; 265: 140-146.
8. **Sousa AS, Sponton ACS, Trifone CB, Delbin MA.** Aerobic Exercise Training Prevents Perivascular Adipose Tissue-Induced Endothelial Dysfunction in Thoracic Aorta of Obese Mice. *Frontiers in Physiology*. 2019; 1-12.
9. **Haberka M, Gasior Z.** Carotid extra-media thickness in obesity and metabolic syndrome: a novel index of perivascular adipose tissue: extra-media thickness in obesity and metabolic syndrome. *Journal of Atherosclerosis.* 2015*;* 239: 169-177.
10. **Haberka M, and Gasior Z.** A carotid extra-media thickness, PATIMA combined index, and coronary artery disease: Comparison with well-established indexes of carotid artery and fat depots. *Journal of Atherosclerosis.* 2015; 243: 307-313.
11. **Haberka M, Sosnowski M, Zuziak-Pruska J, Gasior Z.** Extra-media thickness and epicardial fat: Comparison of a novel carotid artery ultrasound index and a well-established cardiac magnetic resonance fat quantification method. *Journal of Nutrition Metabolism and Cardiovascular Disease.* 2015; 25: 763-770.
12. **Jose SP, Asha S, Im K, Ratheesh M, Santhosh S, Sandya S, B GK, Pramod C.** Nephro-protective effect of a novel formulation of unopened coconut inflorescence sap powder on gentamicin induced renal damage by modulating oxidative stress and inflammatory markers. *Journal of Biomedicine and Pharmacotherapy*. 2017; 85: 128–135.
13. **Ratheesh M, P JS, Asha S, Sandya S, Kumar BG, Krishnakumar IM.** Anti-inflammatory effect of a powder formulation of coconut inflorescence sap against ox-LDL induced inflammatory responses in human peripheral blood mononuclear cells by modulating TLR-NF-ĸB signaling pathway. *Journal of Toxicology Mechanisms and Methods.* 2017: 1-23.
14. **Asgary, S., Afshani, M., Sahebkar, A, et al**. Improvement of hypertension, endothelial function and systemic inflammation following short-term supplementation

with red beet juice: a randomized crossover pilot study. *Journal of Human Hypertension.* 2016;30: 627–632.

15. **Lima EBC, Sousa CNS, Meneses LN, Ximenes NC, Júnior MAS.** Cocos nucifera (L.) (Arecaceae): A phytochemical and pharmacological review. *Brazilian Journal of Medical and Biological Research.* 2015; 48: 953–964.
16. **Skilton MR, Boussel L, Bonnet F, Bernard S, Douek PC, Moulin P, and Serusclat A.** Carotid intima-media and adventitial thickening: comparison of new and established ultrasound and magnetic resonance imaging techniques. *Journal of Atherosclerosis.* 2011*;* 215: 405-410.
17. **Skilton MR, Serusclat A, Sethu AH, Brun S, Bernard S, Balkau B, Moulin P, and Bonnet F.** Noninvasive measurement of carotid extra-media thickness: associations with cardiovascular risk factors and intima-media thickness. *Journal of American Cardiology.* 2009*;* 2: 176-182.
18. **Skilton MR, Sullivan TR, Ayer JG, Garden FL, Harmer JA, Leeder SR, Toelle BG, Webb K, Marks GB, and Celermajer DS.** Weight gain in infancy is associated with carotid extra-medial thickness in later childhood. *Journal of Atherosclerosis.* 2014*;* 233: 370-374.
19. **Skilton MR, Sullivan TR, Ayer JG, Harmer JA, Toelle BG, Webb K, Marks GB, and Celermajer DS.** Carotid extra-medial thickness in childhood: early life effects on the arterial adventitia. *Journal of Atherosclerosis.* 2012; 222: 478-482.
20. **Bortel LM Van, Duprez D, Starmans-kool MJ, et al.** Clinical Applications of Arterial Stiffness, Task Force III: Recommendations for User Procedures. *American Journal of Hematology*. 2002; 15:445–452.
21. **O'Rourke M.** Arterial Stiffness, Systolic Blood Pressure, and Logical Treatment of Arterial Hypertension. *Journal of Hypertension.* 1990; 15:339–347.
22. **Ferreira JP, Girerd N, Bozec E, Machu JL et al.** Intima-media thickness is linearly and continuously associated with systolic blood pressure in a population-based cohort (STANISLAS cohort study). *Journal of the American Heart Association.* 2016; 5: 1-9.
23. **Lehman SJ, Massaro JM, Schlett CL, O'Donnell CJ, Hoffmann U, Fox CS.** Peri-aortic fat, cardiovascular disease risk factors, and aortic calcification: The Framingham Heart Study. *Journal of Atherosclerosis*. 2010; 656–661.
24. **Schulze-Bauer CAJ, Regitnig P, Holzapfel GA.** Mechanics of the human femoral adventitia including, the high-pressure response. *American Journal of Physiological Heart Circulation Physiology*. 2002; 427-440.
25. **Mattace-Raso FUS, Van Der Cammen TJM, Hofman A, et al.** Arterial stiffness and risk of coronary heart disease and stroke: The Rotterdam Study. *Journal of Circulation*. 2006; 113: 657–663.
26. **Gaddum NR, Keehn L, Guilcher A et al.** Altered dependence of aortic pulse wave velocity on transmural pressure in hypertension revealing, structural change in the aortic wall. *Journal of Hypertension*. 2015; 65: 362–369.
27. **Vermeersch SJ, Dynamics B, Society L.** Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors : establishing normal and reference values. *European Heart Journal.* 2010; 31: 2338–2350.
28. **Vriz O, Magne J, Driussi C, et al.** Comparison of arterial stiffness/compliance in the ascending aorta and common carotid artery in healthy subjects and its impact on left ventricular structure and function. *The International Journal of Cardiovascular*

Imaging. 2017; 33:521–31.

29. **Yuan C, Wang J, Ying M.** Predictive Value of Carotid Distensibility Coefficient for Cardiovascular Diseases and All-Cause Mortality : A Meta-Analysis. *PLOS ONE*. 2016;19:1–15.
30. **Cai TY, Sullivan TR, Ayer JA, et al.** Carotid extra-medial thickness is associated with local arterial stiffness in children. *Journal of Hypertension.* 2016; 34: 109-115.
31. **Cai TY, Magnussen C, Haluska B, Skilton MR et al**. Carotid extra-medial thickness does not predict adverse cardiovascular outcomes in high-risk adults. *Journal of Diabetes & Metabolism.* 2016; 42: 200-203.
32. **Sommer G, Regitnig P, Költringer L, Holzapfel GA.** Biaxial mechanical properties of intact and layer-dissected human carotid arteries at physiological and supraphysiological loadings. *Journal of Physiological Circulation of physiology*. 2009; 898–912.
33. **Thijssen DHJ, Scholten RR, Van Den Munckhof ICL, Benda N, Green DJ, Hopman, MTE.** Acute change in vascular tone alters intima-media thickness. *Journal of Hypertension*. 2011; 58: 240–246.
34. **Greenwood SA, Manhahis, E, Castle EM, et al.** Arterial stiffness is a predictor for acute kidney injury following coronary artery bypass graft surgery. *Journal of Cardiothoracic Surgery*. 2019; 14:51.
35. **Safar ME, Levy BI, Struijker-Boudier H.** Current Perspectives on Arterial Stiffness and Pulse Pressure in Hypertension and Cardiovascular Diseases. *Journal of Circulation*. 2003;107: 2864–2869.

Appendix: Grant Proposal

Aging results in an increased risk for cardiovascular disease (CVD) related events (1,2,3,4). Increased age-related events are associated with arterial stiffening independent of traditional risk factors (2,3,4,5). Arterial stiffness is a significant risk factor for cardiovascular disease (CVD) (4,6). Arterial stiffness is defined as the stiffening of the large elastic arteries, including the aorta and carotid artery (4). Changes in blood pressure, smooth muscle function, and endothelial function will begin to over time affect the structure of the artery (1,2). These changes in structure relate to but are not limited to intima-media thickness (IMT), extra-media thickness (EMT) elastin, collagen, smooth muscle cell function, and advanced glycation end products (1,4,6,7,8). EMT is defined as the combination of perivascular adipose tissue (PVAT), the interstitial wall, and arterial adventitia of the carotid artery, and could be essential to understand as increased structural thickness could be related to arterial stiffness and future CVD events (4,6).

A contributing factor toward arterial stiffness is inflammation (1,4). Inflammation contributes to a change in the function of the vasculature and generates structural remodeling (1,4,6,7). Suggesting that inflammation can lead to endothelial dysfunction, which can cause an eventual change in structure (1). Much of these functional issues leading to inflammation are due to lifestyle (1,9,10,11,12). PVAT, as presented by Sousa et al, is directly related to inflammation and oxidative stress as a result of obesity (13). Sousa et al, performed a study of obese and non-obese rats (13). Results demonstrated an increase in endothelial dysfunction and oxidative stress of the obese rats (13). Indicating that an increase in PVAT can increase inflammation (13). Additionally, the rats who performed regular aerobic exercise saw a decrease in inflammation and PVAT dysfunction (13).

Moreover, Fleenor et al, demonstrated with mice who performed habitual aerobic exercise elicited a decrease in arterial stiffness and adventitial collagen (14). Research performed by Sommer et al, demonstrated increases in pressure and decreases in carotid compliance and distensibility from older harvested common carotid arteries (CCA) of deceased humans compared to the younger (15). Indicating inverse relationships between higher pressures and distensibility and compliance of the CCA (15). Comparable results were demonstrated with Schulze-Bauer et al, with stenotic femoral arteries during autopsy (16). The arteries that were stiffer and less compliant had a higher susceptibility toward stiffening (16). What this research indicates is that there is a consistent increase in arterial stiffness and vascular remodeling with aging (1,12-16).

However, it also indicates a possible effect of aerobic or dietary interventions to decrease arterial stiffness and lower CVD risk. COCOZEN is a coconut-derived substance that has been shown to have anti-inflammatory effects (10,11,12). COCOZEN is utilized in other cultures as an anti-inflammatory agent (10,11,12). COCOZEN has limited usage in specific dietary interventions and has not been used as a supplement in a human intervention before and the effect on vascular health is unknown. Additionally, the influence of COCOZEN with age-related stiffness and EMT is unknown. Therefore, the focus of this study is to assess the effects of COCOZEN on arterial stiffness and EMT.

Question

The research question for this study is, what effect does COCOZEN have on EMT and arterial stiffening in middle-aged and older adults? This will add to the scientific literature by eliciting information on COCOZEN's impact on arterial stiffness and EMT. Thus, the purpose of this study will be to determine if adherence to 1.5 grams (g) of COCOZEN over 8-weeks affects arterial stiffness and EMT in middle-aged and older adults. We hypothesize that middle-aged and older adults adhering to an 8-week COCOZEN intervention, will decrease arterial stiffness and EMT. The aims of the study are as follows:

1. To determine the effect of COCOZEN on arterial stiffness and EMT compared to a placebo in middle-aged and older adults.
2. To determine if EMT in middle-aged and older adults is associated with arterial stiffness.

Expected Outcomes

Based on previous literature we would expect that adherence to COCOZEN over the course of the study will decrease arterial stiffness and EMT, and that EMT will be associated with arterial stiffness (13,14,15). The anti-inflammatory benefits provide a rationale that COCOZEN will lower arterial stiffness and EMT (10,11,12).

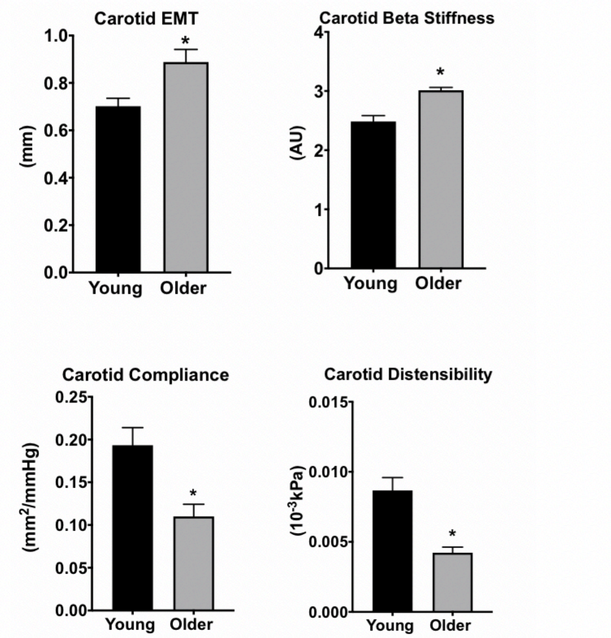
Research Strategy

Arterial stiffness is an established risk factor for CVD (4,6). Structural measures of the vasculature include IMT and EMT (1,17-20). Specific contributing research about EMT, was performed by Skilton et al who examined carotid EMT with ultrasonography in healthy and non-healthy individuals which were defined by the type and number of risks factors (21-24). This study included 175 participants ranging from 21-83 years of age (54 diabetics, 43 with dyslipidemia, 26 with risk factors, and 52 healthy who were free of known disease) who participated (22). Results demonstrated an increased EMT from those who had dyslipidemia and diabetes compared to those who were healthy (22). Representing a linear relationship with EMT and CV risk factors (22). This is significant because increased information about EMT could elicit valuable information to predict CVD risk (5,6).

Background

Ball State University has limited unpublished data from a pilot study investigating EMT. Some of these data are presented in Figure 1. Fleenor et al., studied 21 young and old individuals (young=22-24 years of age and old=45-67 years of age) performing measurements of carotid artery stiffness. Measurements of EMT were acquired as well as carotid beta stiffness defined as Ln (Pmax/Pmin)/[Dmax-Dmin)/Dmin], where P stands for pressure and D is diameter which is located through ultrasound (6,7). Compliance and distensibility are defined as the "ease of distention" with the opposite leading, to a stiffer artery (7,25). Compliance and distensibility are calculated as πDmin*ΔD/2*ΔP and 2*[(ΔD/Dmin)/ΔP] respectively (min=diameter measured in diastole, d=diameter, Δ=change) (7,25,26). Each of these are measures of stiffness and a combination of function and structure (1,7,26). The results demonstrated that the younger individuals had a thinner EMT and greater carotid compliance and distensibility as, well as a lower beta stiffness. Demonstrating a linear relationship between EMT, stiffness, and age.

Figure 1: Comparison of EMT, carotid Beta stiffness, carotid compliance and distensibility in young and old individuals (Fleenor et al).



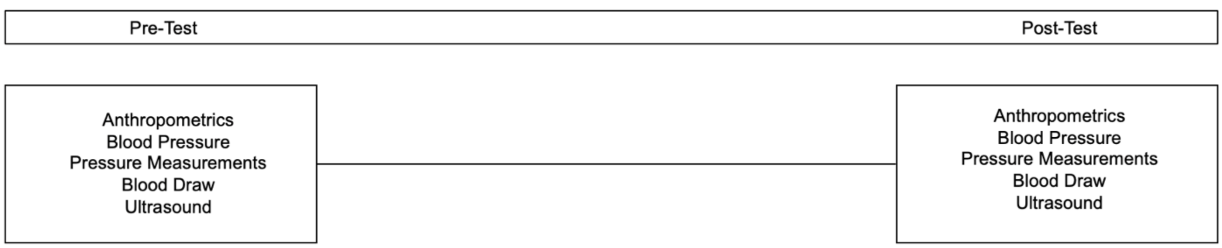
Limited literature has utilized pharmaceuticals as a potential way to help limit inflammatory reactions within the vasculature. In other cultures, coconut-derived products have been utilized as a way to reduce inflammation within animal studies (10,11,12). Previous research of coconut-derived substance has been performed in male Wistar rats. Specifically, Jose et al, examined rats who were of average weight and were given a normal low-fat diet (10). None of the rats had any pre-existing conditions to indicate that they would have increased inflammation prior to the start of the study (10). Conclusions indicated that a coconut-derived substance subdued inflammatory markers related to kidney function in male Wistar rats (10). Ratheesh et al, found similar results but in human peripheral blood mononuclear cells indicating a potential anti-inflammatory effect which, could have an impact on stiffness (10,11). Thus, this provides evidence that an anti-inflammatory supplement could affect arterial stiffness and EMT (10,11,12). However, there are no research findings about EMT and COCOZEN combined in a middle-aged and older adult population. If EMT is proven to be a useful measure in predicting CVD risk factors, it could impact how future vascular research is conducted (27,28).

Innovation

This study is innovative for three reasons. First is that EMT is a novel measurement (19). EMT has rarely been measured and, there is limited evidence about its association towards arterial stiffness and predictive nature of CVD (6,14,21-24). Though there are contributing studies about other vascular structures (i.e., IMT), EMT is unknown (8,21,29). Thus, if this study demonstrates that EMT is correlated to arterial stiffness (5,6). EMT could be an important structure to study to understand further the associations to arterial stiffness and CVD risk (2,3,4).

Second, we will utilize carotid compliance, distensibility, and beta-stiffness calculations utilized through ultrasound and pressure measurements to see how COCOZEN affects arterial stiffness (6,7). There appears to be limited research about carotid compliance, distensibility, and beta-stiffness thus; this research could assist in establishing normal values for these measures of arterial stiffness (30,31). Third, COCOZEN as an anti-inflammatory substance has not been utilized previously in an intervention, thus allowing a greater understanding of its effect on vascular function and structure (10,11,12).

Figure 2: Overview of the Design.



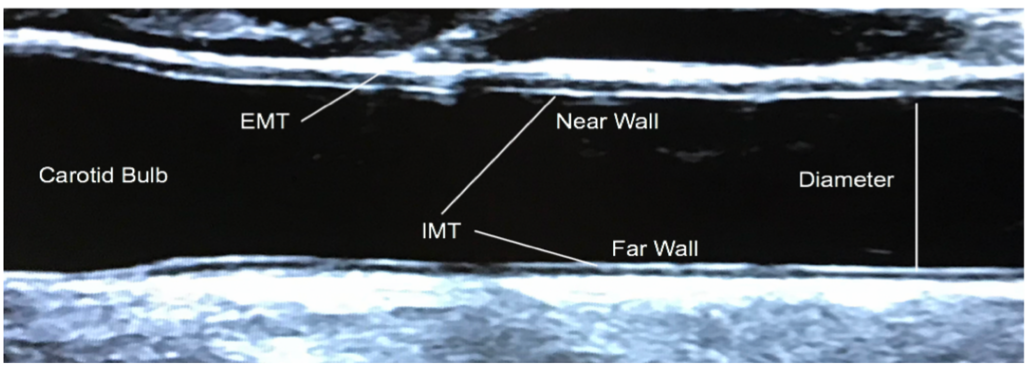
Approach

This study is an 8-week, double-blind, randomized control trial. The objective is to recruit approximately 14 participants within this study. Participants will be recruited based on the following inclusion criterion; between the ages of 40-80 years. Participants will be excluded if they exhibited one or more of the following: a body mass index (BMI) less than 18 kilogram/meters^2 (kg/m^2) or greater than 35 kg/m^2, resting brachial blood pressure of greater than 160/100 millimeters of mercury (mmHg), a current smoker, known clinical disease, an abnormal resting 12-lead electrocardiogram (ECG) previous angina, alcohol dependence or abuse, more than three days of self-reported vigorous activity per week, or self-reported allergies to nuts or coconuts.

This study includes two testing sessions (Figure 2). A pre-test at week 0 and post-test at week 8. Participants will be randomly assigned to a group after qualifying for participation. The pre-test will include an informed consent and health history questionnaire. Participants will be instructed to refrain from caffeine, alcohol, and exercise 24 hours prior to the pre-test and will be required to perform an overnight fast. The supplement or placebo will be administered in a 28-day pill tray and given to each participant. Three pills at 500 milligrams (mg) each (total 1500 mg) per day will be taken and recorded on a log sheet. Baseline measurements will include basic anthropometrics, such as height, weight, and body fat percentage measured with dual-energy X-ray absorptiometry (DEXA). Additionally, a blood draw containing a full lipid profile and glucose will be conducted. As well as brachial, carotid and radial pressure measurements. All anthropometric measures were standardized following the American College of Sports Medicine (ACSM) (5).

Participants will be seated approximately for five-minutes before brachial blood pressure will be measured two times and averaged (5). Thereafter, carotid blood pressure measurements at the carotid and radial pulse will be performed and conducted by the same technician with a SphygmoCor system tonometer (6). Participants will be instructed to lie down in the supine position for approximately 10 minutes (6). Upon which, the pressure waveforms measurements will be obtained on the left radial pulse and left common carotid artery (LCCA) (6). Ultrasound will be performed in the supine position thereafter, the participant will be attached to a three-lead ECG, and a longitudinal measurement of the right common carotid artery (RCCA) will be performed (Figure 3). The post-test at week 8 follows the same procedures as the pre-test. To reduce variability, the same technician will perform the ultrasound and pressure measurements.

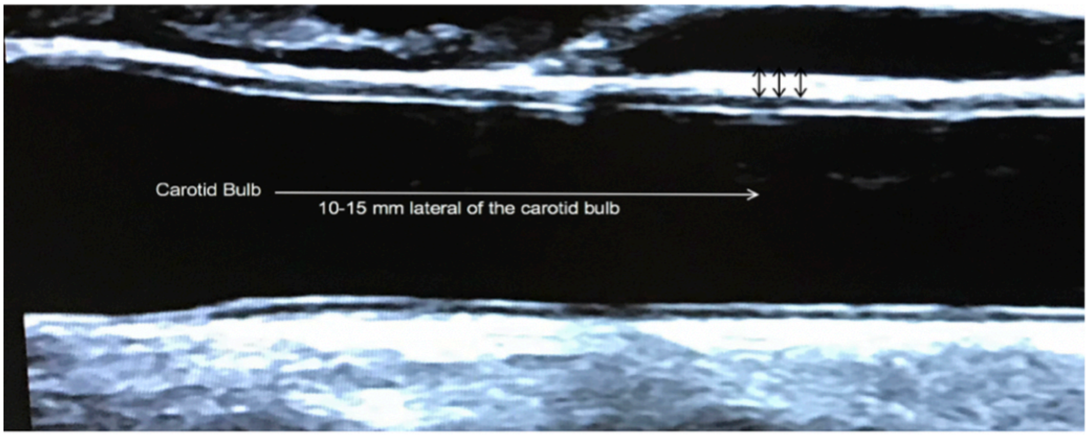
Figure 3: Longitudinal view of the right common carotid artery (RCCA) using Doppler ultrasound with a General Electric (GE)® system (Lefferts et al, Skilton et al).



After all data are collected, data analysis is conducted. The specific standardization procedures are separated into carotid stiffness measures and EMT. Stiffness measures are calculated with the formulas as demonstrated above (6,7). EMT will be measured on the QRS complex during diastole approximately 10-15 mm proximal of the carotid bulb as seen on Figure 4 (6, 21-24). Afterward, manual measurement of the EMT will be performed nine times over three cardiac cycles. Finally, IMT, and diameter are measured through an automated software titled CV suite.

Statistical analysis will be a 2-way repeated measures analysis of variance (ANOVA) over the pre and post, and a correlation at baseline, with statistical power and p-value set at $p \leq 0.05$ with an effect size of 0.8. Meaning there is a need of approximately 14 total participants (7 per group) for this power to be achieved. With this knowledge and standardizations in place, the procedure enables the aims of the study to be achieved as a way to understand COCOZEN's effects on EMT and arterial stiffness.

Figure 4: Measurement of Carotid EMT (Skilton et al).



Significance

The significance of this study design is to determine if age related arterial stiffness is associated with concomitant increases in EMT. It is known that arterial stiffness is a precursor for CVD events (1,5). Thus, the importance of understanding what directly causes arterial stiffness as well as contributions to stiffening of the arteries is beneficial as a means to extrapolate more information about how best to prevent a CV event from occurring. Thus, if increased EMT is correlated toward arterial stiffness this could implicate that preventing the thickening of EMT could prove to be beneficially to lower CV risk (13,21-24). Additionally, if COCOZEN impacts arterial stiffness and or EMT due to a reduction in inflammation this too, could provide valuable information to ultrasonographers to measure EMT as a way to see if there is an increased risk for an event due to the thickening of the EMT (1,6). Thus, if this study elicits information to suggest that there is a relationship between EMT and arterial stiffness, this could contribute valuable information about EMT correlation with CVD risk (1,2,3,4,5,6).

Alternative Explanations

Possible explanations of why there may be no effect of COCOZEN on arterial stiffness and EMT could be due to three reasons. The first, may be due to a low sample size. I will attempt to recruit 14 total participants for my research, which is a small sample. Thus, a possible lack of effect on the results could be due to a small sample which would prevent significance to be found. Second, a possible reason for no change could be due to the fact that EMT is not directly associated to stiffness or inflammation in middle-aged and older adults. Thirdly, COCOZEN as a supplement while has been tested to have "anti-inflammatory" properties either may not be effective, may not be enough of a dosage compared to previous literature, or our population may not have enough systemic inflammation to see a response (10,11,32). Ideally, a larger sample would be a beneficial way to increase the power of the study. Though the unexpected findings were not as hypothesized this could demonstrate different significance in other areas that were measured but were not the main outcomes of the study.

Personal Narrative

Since 2014, I studied exercise science and learned specific strategies to apply basic principles and techniques to research methods. During my undergraduate work at Taylor University, I completed a research methods class that included the development and implementation of a research study. This study examined the effect of supplementation on resistance training. More specifically, the study examined the effect of a pre-workout supplement on moderately trained males on 1-rep max in the bench press and box squat. The objectives of the class assignment were 1) to gain an understanding of the research process by developing and conducting a research study, and 2) to implement the methods and tools of research. The study provided the opportunity to experience the complete research process. For example, I developed an Institutional Review Board (IRB) proposal. Thus, my research experience started before graduate school. Additionally, I practiced and became proficient at the techniques involved in this COCOZEN study. These include blood draws, blood pressure and doppler ultrasound. Finally, I have been working with a variety of participants at the Adult Physical Fitness Program at Ball State University and Ball Memorial Hospital. These experiences prepared me to participate and contribute to this research study.

Literature Review

Table 1: Extra-medial thickness, Arterial Stiffness, and Cardiovascular Risk Factors				
Study	**Purpose**	**Sample**	**Study Design**	**Results**
Cai et al, (2016)	-Structural changes may be associated with arterial stiffening. -It is hypothesized that children with increased EMT would have increased arterial stiffness.	-248, 8-year-old children.	-Observational. -Measures of arterial stiffness included Beta-stiffness index, distensibility index, and incremental modules of elasticity.	-Carotid EMT is correlated with all three measures of arterial stiffness (p=0.02, p=0.03, p=0.05). -The results indicate that carotid EMT is associated with stiffness and specifically the arterial adventitia could be correlated with arterial stiffness during childhood.
Skilton et al, (2009)	-Measurement of carotid EMT with ultrasonography. -The focus of this study was looking at vascular health in healthy, and non-healthy individuals.	-175 subjects (54 diabetic, 43 dyslipidemia, 26 other CVD risk factors, 52 healthy.	-Observational.	-EMT was increased from those who were diabetic and with dyslipidemia (p<0.001, 0.04) compared to the healthy individuals. Demonstrating a linear relationship with EMT and cardiovascular risk factors.
Skilton et al, (2011)	-Carotid IMT is a non-invasive marker of cardiovascular disease. -To determine the effect of IMT and adventitia with ultrasound on carotid wall thickness in heathy individuals.	-Carotid ultrasound and magnetic resonance imaging in 20 participants.	-Observational. -To see effects of vascular measurements on healthy individuals.	-Results included ultrasound-derived measures of arterial wall thickness, which were highly correlated with wall thickness (p<0.001). -The EMT contributed greater to information concerning vascular structures than IMT alone.
Skilton et al, (2012)	- The purpose of this study to was to determine associations between EMT and CVD risk factors in younger children.	-389, 8-year old children were analyzed with high-resolution ultrasound all of which were non-diabetic.	-Observational. -389 participants were analyzed with ultrasound by EMT thickness.	-EMT was lower in girls and was closely correlated with blood pressure. The same remained true for IMT. -Thicker EMT is associated with gender in height in younger children. Indicating that EMT may give useful information about early arterial health.
Skilton et al, (2014)	-The purpose was to determine if fetal growth and postnatal growth is connected with EMT in children.	-Ultrasound analysis with 379 non-diabetic children was performed.	-Observational. -Carotid EMT was measured with ultrasonography	-Weight gain for birth to 2.5 years was associated with EMT. This association was higher in boys than girls.

Summary: Based on the literature there appears to be associations between EMT and arterial stiffness across all age groups. This allows for repeatable research to be performed.

Table 2 Part A: Extra-media thickness, and Measures of Arterial Stiffness				
Study	**Purpose**	**Sample**	**Study Design**	**Results**
Haberka et al, (2015)	-Determining associations between EMT thickness, obesity, and metabolic syndrome. As well as CV risk factors.	-Four hundred patients were included in the study (age: 60.95 ± 7.3 years, Female/Male: 35/65%).	-Observational. -Both the common carotid arteries and EMT/IMT were measured, along with body fat, and anthropometric measurements.	-EMT for those with metabolic syndrome was found thicker and was correlated with fat distribution and general obesity ($p < 0.001$). -This indicates there is a relationship between EMT and metabolic syndrome.
Haberka et al, (2015)	-The goal was to determine associations between periarterial fat, EMT and CAD in comparison with patients in high and very high CVD risk categories.	Four hundred twenty-two patients scheduled for elective coronary angiography were included in the study (age: 61.3 ± 7.4 years; males 65%).	-Observational -EMT and IMT as well as epicardial and pericardial fat thickness were measured. These participants had an estimated cardiovascular risk of 82% and 60%.	-For patients with CAD and critical coronary stenosis there was higher EMT compared to the control groups. -The combination of ultrasound indexes related to periarterial fat and vascular wall (PATIMA index) is associated with more complex CAD in high and very-high risk patients.
Haberka et al, (2015)	-The purpose was to evaluate the association between carotid EMT and epicardial fat and pericardial fat and their association to cardiovascular risk and metabolic syndrome.	-One hundred consecutive patients (age: 51.7 ± 15.4 years; males 70%) scheduled for cardiac magnetic resonance were prospectively included in the study.	-Observational.	-Higher risk subjects demonstrated associations with increased EMT. Carotid EMT may be a good surrogate marker, of both periarterial fat and arterial adventitia.
Lefferts et al, (2017)	-The purpose was to determine associations of Carotid stiffness, EMT, and visceral adiposity in healthy young adults.	-135 healthy males (20 ± 2 years, body mass index [BMI] 24.8 ± 3.3 kg/m^2).	-Observational design. Two separate measurements on different days included vascular, and anthropometric. Brachial and carotid systolic, diastolic, and pulsatile blood pressure were measured with oscillometric cuff and applanation tonometry.	-Carotid PP and EMT are correlated to Beta-stiffness. Results suggest visceral adiposity could change markers of CVD and could contribute to arterial stiffness. -Formula for Beta-stiffness: Ln $(P_{max}/P_{min})/[D_{max}-D_{min})/D_{min}]$.

Summary: EMT thickness is related to CAD and CVD risk factors. Additionally, research was gathered on Beta-stiffness and ultrasonography to allow for repeatability.

Table 2 Part B: Extra-media thickness, and Measures of Arterial Stiffness				
Lyle et al, (2017)	-Overview of some of the most common mechanisms that contribute to increased arterial stiffness.	-Various subjects with different clinical risk factors.	-Review article.	-Arterial stiffness is accelerated with various risk factors. Some of which include TD2. Additionally, arterial stiffness is a precursor for many common risk factors.
Vriz et al, (2017)	-To compare arterial stiffness/compliance in the aorta and common carotid artery and its effect on left ventricular structure and function.	-548 healthy subjects.	-Observational.	-Common carotid artery stiffness parameters were correlated with age and systolic blood pressure. -Common carotid stiffness measures were better predictors of left ventricular structure and function than aortic stiffness.
Yuan et al, (2016)	-The purpose was to determine the predictive value of carotid distensibility coefficient for cardiovascular diseases and all-cause mortality.	-20,361 subjects over 11 longitudinal studies.	-Meta-analysis.	-Carotid distensibility coefficient predicted future cardiovascular events and all cause-mortality. -This may help diagnosis of high-risk patients in treatment of cardiovascular diseases.

Summary: This demonstrates that unique measures of stiffness are associated to CVD risk.

Table 3: Arterial Stiffness and Mortality				
Study	**Purpose**	**Sample**	**Study Design**	**Results**
Greenwood et al, (2019)	-PWV noninvasive tool to measure stiffness. PWV is hypothesized to predict kidney injury.	37 patients were included in the study. 85% were male, and mean age was 66.3 years (SD = 9.7 years).	-Observational.	-1.5 greater chance of kidney injury with higher PWV. -Independently predicts acute kidney injury.
Mattace-Raso et al, (2006)	-Determining if arterial stiffness is a risk for coronary artery disease and stroke in healthy subjects.	-The present study included 2835 subjects participating in the third examination phase of the Rotterdam Study.	-Longitudinal, examined follow-up of 4.1 years. - “Arterial stiffness was measured as aortic pulse wave velocity and carotid distensibility.”	-CAD risk was found to be greater in those with higher PWV. -Aortic PWV was found to be an independent risk factor for stroke and CAD in healthy adults.

Summary: Associated information about PWV and CVD risk, which solidifies strong associations between the two.

Table 4: Arterial stiffness, Pressure Components and Associations for CVD risk				
Study	**Purpose**	**Sample**	**Study Design**	**Results**
Bortel et al, (2002)	-Information about arterial stiffness and pressure components and its clinical applications.	-n/a	-n/a	-Arterial stiffness is related to distensibility and compliance, explained through these equations: Carotid Compliance: $\pi Dmin*\Delta D/2*\Delta P$ and Carotid Distensibility: $2*[(\Delta D/Dmin/\Delta P]$
Gaddum et al, (2015)	-A novel technique is used to modulate intrathoracic pressure and aortic transmural pressure in normotensive and hypertensive individuals.	-n=20; age, 52.1±15.3 years (hypertensive) -n=20; age, 55.5±11.1 years. (normotensive)	-Experimental. -Doppler probes, and PWV.	-PWV velocity was higher in hypertensive individuals. -Hypertension is related to elastin and wall/diameter changes.
Lehman et al, (2010)	-Perivascular fat may play a role in obesity mediated vascular disease.	-(n=1067, mean age 59 years, 56.1% women), thoracic peri-aortic fat depots were quantified. Visceral abdominal tissue and calcification of the thoracic and abdominal aorta were also measured.	-Observational.	-Thoracic peri-aortic fat is associated with measures of adiposity, metabolic risk factors, and coronary and abdominal aortic calcification.
Mitchell et al, (2010)	To determine cardiovascular risk makers in relation to PWV.	-2232 total participants all part of the Framingham Heart Study.	-Observational, with a mean follow up of 0.2-8.9 years.	-Results demonstrated that 6.8% had an event (151/2232) and higher aortic PWV was associated with 48% increase risk of CVD.

Summary: Focus of functional and structural components of the vasculature and how they are measured and related to CVD.

Table 5: EMT Stucture Breakdown (peivascular adipose tissue, arterial adventitia, and interstitial wall)				
Study	**Purpose**	**Sample**	**Study Design**	**Results**
Fleenor et al, (2010)	-They tested the hypothesis that carotid artery stiffening with aging is associated with changes in vascular structures and could be reversed with aerobic exercise.	-Twenty young (4–7 months) and 20 old (29–32 months) male B6D2F1 mice	-Experimental.	-Increases in carotid artery stiffness with aging are associated with TGF-B1 stimulated increase in adventitial collagen I and III related to changes in fibroblasts. With aerobic training many of these changes can be delayed or reduced.
Schulze-Bauer et al, (2002)	-Inflation tests of 11 non-stenotic human femoral arteries were performed during autopsy.	-Eleven human femoral arteries (79.3±8.2 years, means ± SD) were investigated within 24 hours from death.	-Observational.	-Those arteries that were stiffer were not as efficient at dilation and constriction as well as those who had excess pressure were more susceptible to hardening.
Sommer et al, (2010)	-To enhance the knowledge of the mechanical functions of arteries and their associated layers in specific pathophysiological and clinical problems, such as hypertension and angioplasty with stenting.	-Eleven human CCAs and 10 human ICAs were harvested from 11 human subjects ranging from 67 to 86 years, 4 women and 7 men).	-Observational.	-Negative correlations were found with age and axial inversion stretches for the CCA's. Due to aging being related to a decrease in distensibility, structural changes and loss of elastin fibers.
Sousa et al, (2019)	-The present study aimed to investigate the effects of aerobic exercise training on PVAT-induced endothelial dysfunction of thoracic aorta of obese mice.	-Male sedentary and obese rats.	-Observational.	-The PVAT-induced endothelial dysfunction in obese rats was associated with circulatory inflammation and oxidative stress. Aerobic exercise training upregulated the antioxidant expression and decreased PVAT oxidative stress with a beneficial impact on endothelium-dependent relaxation.

Summary: Structure of the artery and its potential of reversal and relation to oxidative stress. Reversal of the adventitia is demonstrated with exercise.

Table 6: Coconut-Derived Substances and Beet Juice on Cardiovascular Risk				
Study	**Purpose**	**Population**	**Study Design**	**Results**
Asgary et al, (2016)	To investigate the effect of raw beet juice and cooked beet juice on blood pressure in hypertensive subjects.	-24 hypertensive subjects (25-68 years old).	-Randomized crossover.	-Overall, both forms of beet root juice improved blood pressure, endothelial function, and systemic inflammation, however, the raw beet juice had greater anti-hypertensive effects.
Jose et al, (2017)	-To see the effect of a coconut derived supplement on inflammatory makers (such as kidney function) in rats. Which is hypothesized to have great health benefits.	-Adult male Wistar rats.	-Experimental randomized control. -The animals were grouped into three groups one with vehicle, one with gentamicin and one with gentamicin +CSP for 16 days.	-Coconut flower sap has a great potential to prevent or reverse kidney damage. -CSP, demonstrated many anti-inflammatory markers of kidney function contributing to the hypothesis of CSP to have great anti-inflammatory health benefits.
Lima et al, (2015)	-To review the effect Cocos nucifera has on phytochemical, pharmacological and toxicological aspects in humans and to guide future clinical research.	-n/a	-Review article	-Cocos nucifera demonstrated positive effects on vascular health but varies depending on the part of the plant studied.
Ratheesh et al, (2017)	-To determine the ant-inflammatory effects of a coconut-derived substance on LDL and atherosclerosis.	-Healthy human volunteers in three different groups.	-Experimental Design. -Human peripheral blood mononuclear cells were isolated between cultured groups (1,2,3) and analyzed for inflammatory markers.	-The use of the coconut substance helped decrease inflammatory effects on human peripheral blood mononuclear cells.

Summary: Anti-inflammatory substances can reduce inflammation and functional measures of the vasculature.

Abbreviations: EMT, extra-medial thickness, IMT, intima-medial thickness, CVD, cardiovascular disease, CAD, coronary artery disease, %, percent, PATIMA, periarterial index, BMI, body mass index, kg, kilograms, m, meters, PP, pulse pressure, D, diameter, Δ, change, P, pressure, min, minimum, max, maximum, TD2, type-2 diabetes, PWV, pulse wave velocity, SD, standard deviation, PVAT, perivascular adipose tissue, CCA, common carotid artery, ICA, internal carotid arteries, TGF- β1, transforming growth factor-β1, LDL, low-density lipoprotein, CSP, coconut flower sap.

References

1. **Fleenor BS, Berrones AJ.** *Arterial Stiffness: Implications and Interventions*. Springer Briefs in Physiology. 2015: 1-67.
2. **Lyle AN, Raaz U, et al.** Killing me un-softly: Causes and mechanisms of arterial stiffness Recent Highlights of ATVB: *Journal of Arteriosclerosis Thrombosis Vascular Biology*. 2017; 37:1–22.
3. **Mattace-Raso FUS, Van Der Cammen TJM, Hofman A, et al.** Arterial stiffness and risk of coronary heart disease and stroke: The Rotterdam Study. *Journal of Circulation*. 2006*;* 113: 657–663.
4. **Mitchell GF, Hwang S, Vasan RS, et al.** Arterial stiffness and cardiovascular events: The Framingham Heart Study. *Journal of Circulation*. 2011; 121:505–511.
5. **Riebe D, Ehrman J, Liguori G, Magal M.** *ACSM's Guidelines for Exercise Testing and Prescription*. 10th ed. Wolters Kluwer; 2016: 1-451.
6. **Lefferts WK, Sperry SD, Jorgensen RS, Kasprowicz AG, Skilton MR, Figueroa A, and Heffernan KS.** Carotid stiffness, extra-media thickness, and visceral adiposity in young adults. *Journal of Atherosclerosis.* 2017; 265: 140-146.
7. **Bortel LM Van, Duprez D, Starmans-kool MJ, et al.** Clinical Applications of Arterial Stiffness, Task Force III: Recommendations for User Procedures. *American Journal of Hematology*. 2002; 15:445–452.
8. **Thijssen DHJ, Scholten RR, Van Den Munckhof ICL, Benda N, Green DJ, Hopman MTE.** Acute change in vascular tone alters intima-media thickness. *Journal of Hypertension*. 2011; 58: 240–246.
9. **Gaddum NR, Keehn L, Guilcher A, et al.** Altered dependence of aortic pulse wave velocity on transmural pressure in hypertension revealing structural change in the aortic wall. *Journal of Hypertension*. 2015; 65: 362–369.
10. **Jose SP, Asha S, Im K, Ratheesh M, Santhosh S, Sandya S, B GK, Pramod C.** Nephro-protective effect of a novel formulation of unopened coconut inflorescence sap powder on gentamicin induced renal damage by modulating oxidative stress and inflammatory markers. *Journal of Biomedicine and Pharmacotherapy.* 2017; 85: 128–135.
11. **Ratheesh M, P JS, Asha S, Sandya S, Kumar BG, Krishnakumar IM**. Anti-inflammatory effect of a powder formulation of coconut inflorescence sap against ox-LDL induced inflammatory responses in human peripheral blood mononuclear cells by modulating TLR-NF-κB signaling pathway. *Journal of Toxicology Mechanisms and Methods.* 2017: 1-23.
12. **Lima EBC, Sousa CNS, Meneses LN, Ximenes NC, Júnior MAS.** Cocos nucifera (L.) (Arecaceae): A phytochemical and pharmacological review. *Brazilian Journal of Medical and Biological Research.* 2015; 48: 953–964.
13. **Sousa AS, Sponton ACS, Trifone CB, Delbin MA.** Aerobic Exercise Training Prevents Perivascular Adipose Tissue-Induced Endothelial Dysfunction in Thoracic Aorta of Obese Mice. *Frontiers in Physiology*. 2019; 1-12.
14. **Fleenor BS, Marshall KD, Durrant JR, Lesniewski LA, Seals DR.** Arterial stiffening with ageing is associated with transforming growth factor- β 1-related changes in adventitial collagen : reversal by aerobic exercise. *Journal of Physiology*. 2010; 20: 3971–82.

15. **Sommer G, Regitnig P, Költringer L, Holzapfel GA.** Biaxial mechanical properties of intact and layer-dissected human carotid arteries at physiological and supraphysiological loadings. *Journal of Physiological Circulation of physiology*. 2009; 898–912.
16. **Schulze-Bauer CAJ, Regitnig P, Holzapfel GA.** Mechanics of the human femoral adventitia including the high-pressure response. *American Journal of Physiological Heart Circulation Physiology*. 2002: 427-440.
17. **Cai TY, Sullivan TR, Ayer JA, et al.** Carotid extra-medial thickness is associated with local arterial stiffness in children. *Journal of Hypertension.* 2016; 34: 109-115.
18. **Haberka M, Gasior Z.** Carotid extra-media thickness in obesity and metabolic syndrome: a novel index of perivascular adipose tissue: extra-media thickness in obesity and metabolic syndrome. *Journal of Atherosclerosis.* 2015; 239: 169-177.
19. **Haberka M, and Gasior Z.** A carotid extra-media thickness, PATIMA combined index, and coronary artery disease: Comparison with well-established indexes of carotid artery and fat depots. *Journal of Atherosclerosis.* 2015; 243: 307-313.
20. **Haberka M, Sosnowski M, Zuziak-Pruska J, Gasior Z.** Extra-media thickness and epicardial fat: Comparison of a novel carotid artery ultrasound index and a well-established cardiac magnetic resonance fat quantification method. *Journal of Nutrition Metabolism and Cardiovascular Disease.* 2015*;* 25: 763-770.
21. **Skilton MR, Boussel L, Bonnet F, Bernard S, Douek PC, Moulin P, and Serusclat A.** Carotid intima-media and adventitial thickening: comparison of new and established ultrasound and magnetic resonance imaging techniques. *Journal of Atherosclerosis.* 2011*;* 215: 405-410.
22. **Skilton MR, Serusclat A, Sethu AH, Brun S, Bernard S, Balkau B, Moulin P, and Bonnet F.** Noninvasive measurement of carotid extra-media thickness: associations with cardiovascular risk factors and intima-media thickness. *Journal of American Cardiology.* 2009*;* 2: 176-182.
23. **Skilton MR, Sullivan TR, Ayer JG, Garden FL, Harmer JA, Leeder SR, Toelle BG, Webb K, Marks GB, and Celermajer DS.** Weight gain in infancy is associated with carotid extra-medial thickness in later childhood. *Journal of Atherosclerosis.* 2014*;* 233: 370-374.
24. **Skilton MR, Sullivan TR, Ayer JG, Harmer JA, Toelle BG, Webb K, Marks GB, and Celermajer DS.** Carotid extra-medial thickness in childhood: early life effects on the arterial adventitia. *Journal of Atherosclerosis.* 2012*;* 222: 478-482.
25. **O'Rourke M.** Arterial Stiffness, Systolic Blood Pressure, and Logical Treatment of Arterial Hypertension. *Journal of Hypertension.* 1990; 15:339–347.
26. **Greenwood SA, Manhahis, E, Castle EM et al.** Arterial stiffness is a predictor for acute kidney injury following coronary artery bypass graft surgery. *Journal of Cardiothoracic Surgery*. 2019; 14: 51.
27. **Safar ME, Levy BI, Struijker-Boudier H.** Current Perspectives on Arterial Stiffness and Pulse Pressure in Hypertension and Cardiovascular Diseases. *Journal of Circulation*. 2003;107: 2864–2869.
28. **Lehman SJ, Massaro JM, Schlett CL, O'Donnell CJ, Hoffmann U, Fox CS.** Peri-aortic fat, cardiovascular disease risk factors, and aortic calcification: The Framingham Heart Study. *Journal of Atherosclerosis*. 2010; 656–661.
29. **Ferreira JP, Girerd N, Bozec E, Machu JL, et al**. Intima-media thickness is linearly

and continuously associated with systolic blood pressure in a population-based cohort (STANISLAS cohort study). *Journal of the American Heart Association.* 2016; 5: 1-9.

30. **Vriz O, Magne J, Driussi C, et al.** Comparison of arterial stiffness/compliance in the ascending aorta and common carotid artery in healthy subjects and its impact on left ventricular structure and function. *The International Journal of Cardiovascular Imaging*. 2017;33:521–31.
31. **Yuan C, Wang J, Ying M.** Predictive Value of Carotid Distensibility Coefficient for Cardiovascular Diseases and All-Cause Mortality : A Meta-Analysis. *PLOS ONE.* 2016*;* 19:1–15.
32. **Asgary S, Afshani M, Sahebkar A, et al.** Improvement of hypertension, endothelial function and systemic inflammation following short-term supplementation with red beet juice: a randomized crossover pilot study. *Journal of Human Hypertension.* 2016*;* 30: 627–632.